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I, ANNA MAIJA EVERETT, ACTING TEAM LEADER EXAMINATION SUPPORT & SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PQ 1376 for a patent by JAMES BABER ROWE and R.A.M. AL JASSIM filed on 02 July 1999.

I further certify that the above application is now proceeding in the name of UNIVERSITY OF NEW ENGLAND pursuant to the provisions of Section 113 of the Patents Act 1990.

COMMONWEALTH OF AUSTRALIA
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WITNESS my hand this
Nineteenth day of July 2000

A. M. Everett.

ANNA MAIJA EVERETT
ACTING TEAM LEADER
EXAMINATION SUPPORT & SALES

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PROVISIONAL SPECIFICATION FOR THE INVENTION ENTITLED:

Control of Acidosis

AUSTRALIAN PATENT OFFICE SEC ~~113~~

Name and Address of Applicant: The University of New England ~~James Baber Rowe, of 411 Rockvale Road~~, Armidale, New South Wales, 2350, AUSTRALIA; ~~R.A.M. Al Jassim, of 3 Cotterell Place, Armidale, New South Wales, 2350~~, ~~AUSTRALIA~~

Name(s) of Inventor(s): James Baber Rowe and R.A.M. Al Jassim

This invention is best described in the following statement:

Megasphera elsdenii and/or *Selenomonas ruminantium* and have been administered in the feed.

Accordingly, there is a need to provide alternative means of controlling fermentative acidosis.

The three main bacterial species and strains identified in the present invention, *Selenomonas ruminantium, Clostridium*-like *vitulinus,* and *Streptococcus equinus*, for use in the control and detection of acidosis have not previously been considered to be important organisms in the development of fermentative acidosis. In fact in the case of *Selenomonas ruminantium* it should be noted that the isolate selected by Leedle (1970) was identified on the basis of its ability to utilise lactic acid, rather than produce it, and is therefore very different to the isolates that form part of the present invention. All of the microorganisms described in the present invention have the ability to produce lactic acid in the digestive tract of vertebrate animals.

The present invention has identified a series of microorganisms responsible for the development of acidosis and describes vaccines, compositions and methods for the treatment and/or prevention and/or detection of acidosis in animals.

Detailed Description of the Invention

1. Microorganisms involved in acidosis

According to a first embodiment of the invention, there is provided an isolated and purified culture of at least one microorganism capable of producing fermentative lactic acid within the gut of a vertebrate, wherein said microorganism is selected from the group consisting of: *Clostridium*-like species, *Streptococcus* species and *Selenomonas* species.

Typically, the lactic acid producing microorganism of the present invention forms part of the normal gut flora of a vertebrate. More typically, the microorganism is involved in the aetiology of fermentative lactic acidosis in vertebrates. Yet more typically, the microorganism is selected from the group consisting of: *Streptococcus equinus*, *Clostridium*-like *vitulinus*, *Selenomonas ruminantium* and *Streptococcus bovis*. Still more typically, the microorganism is one of the following strains of *Clostridium*-like *vitulinus* (LVR1, LVR4); *Streptococcus equinus* (SER1, SER2); *Selenomonas ruminantium* (SRR1, SRR3) or *Streptococcus bovis* (SbR1). Yet even still more typically, the microorganism is one of the following strains deposited with

fragment or fragments thereof, together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

Typically, the pharmaceutical composition may comprise a combination of two or more of the microorganisms outlined in accordance with the first embodiment of the invention.

Typically, the microorganism is provided as live cells, attenuated cells, killed whole cells, cell lysate, crude antigen mixture or purified antigen or antigens from the microorganism. More typically, the microorganism and/or fragment or fragments thereof, is present as outer membrane and associated proteins of said microorganism. Even more typically, the fragment or fragments of the microorganism is present as an immunogenic polypeptide or glycopeptide, or the like.

Typically, the microorganism present in the pharmaceutical composition may exist as a monoculture of at least one microorganism in accordance with the first embodiment of the invention, or may be present as a mixed culture, wherein the predominant microorganism(s) is that defined in accordance with the first embodiment of the invention.

Typically, the pharmaceutical composition in accordance with the second embodiment of the invention may also include cytokines, such as: G-CSF, GM-CSF, interleukins or tumour necrosis factor alpha, used singly or in combination.

Typically, the pharmaceutical composition in accordance with the second embodiment of the invention may also include an adjuvant. More typically, the adjuvant is selected from the group consisting of: Freunds Complete/Incomplete Adjuvant, Montenide Marcol Adjuvant, Phosphate Buffered Saline and Mannan oil emulsions, saponins (QuilA), dextran (dextran sulphate, DEAE-Dextran), aluminium compounds (Imject Alum), N-acetylglucosamiyl-N-acetylmuramyl-L-alanyl-D-isoglutamine (Gerbu adjuvant). More typically, the adjuvant is selected from the group as described in the Vaccine 1995, vol 13 p 1203; 1993 vol 11 p 293; and 1992 vol 10 p 427, the disclosure of which is incorporated herein by reference.

According to a third embodiment of the invention, there is provided a vaccine, wherein said vaccine comprises at least one of the isolated and purified microorganisms in accordance with the first embodiment of the invention and/or

of the invention and/or fragment or fragments thereof, or a pharmaceutical composition in accordance with the second embodiment of the invention, or a vaccine in accordance with the third embodiment of the invention.

Typically, the microorganism(s) and/or fragment or fragments thereof as administered in accordance with the fourth embodiment of the invention, is administered together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

Typically, the microorganism(s) and/or fragment or fragments thereof as administered in accordance with the fourth embodiment of the invention, may also be simultaneously or sequentially administered with cytokines, such as: G-CSF, GM-CSF, interleukins or tumour necrosis factor. Cytokines can also be combined with adjuvants to enhance the immune response.

According to a fifth embodiment of the invention, there is provided at least one of the isolated and purified microorganisms as defined in accordance with the first embodiment of the invention and/or fragment or fragments thereof, or a pharmaceutical composition as defined in accordance with the second embodiment of the invention, or a vaccine as defined in accordance with the third embodiment of the invention, when used in inducing an immune response against lactic acidosis in a vertebrate.

According to a sixth embodiment of the invention, there is provided the use of at least one of the isolated and purified microorganisms as defined in accordance with the first embodiment of the invention and/or fragment or fragments thereof, or a pharmaceutical composition as defined in accordance with the second embodiment of the invention, in the preparation of a vaccine for inducing an immune response against lactic acidosis in a vertebrate.

According to a seventh embodiment of the invention, there is provided a method for inducing an immune response against lactic acidosis in a vertebrate, comprising administering to said vertebrate an immunologically effective amount of the vaccine as defined in accordance with the third embodiment of the invention.

According to an eighth embodiment of the invention, there is provided a vaccine as defined in accordance with the third embodiment of the invention when used in inducing an immune response against lactic acidosis in a vertebrate.

a vertebrate, when used in the treatment and/or prophylaxis of lactic acidosis in a vertebrate in need of said treatment and/or prophylaxis, wherein said lactic acid is produced by at least one of the isolated and purified microorganisms as defined in accordance with the first embodiment of the invention.

According to a fourteenth embodiment of the invention, there is provided use of an active agent capable of preventing or controlling lactic acid accumulation in the gut of a vertebrate, in the preparation of a medicament for the treatment and/or prophylaxis of lactic acidosis in a vertebrate in need of said treatment and/or prophylaxis, wherein said lactic acid is produced by at least one of the isolated and purified microorganisms as defined in accordance with the first embodiment of the invention.

The following features relate to the twelfth, thirteenth and fourteenth embodiments of the invention.

Typically, the active agent is active against at least one of the isolated and purified microorganisms as defined in accordance with the first embodiment of the invention.

Typically, the active agent is selected from the group consisting of: antibiotics, enzyme preparations, clay preparations, compounds which slow the digesta flow, prebiotics and probiotics.

Typically, the antibiotic is an antibiotic active against lactic acid producing bacteria that can be selected from any listing of antibiotic compounds such as available in text books, and reports such as the draft JETACAR report on antibiotic usage in Australia, the disclosure of which is incorporated herein by reference.

More typically, the antibiotic is selected from the group consisting of: Acyclovir (Zovirax), Amantadine (Symmetrel), Amikacin (generic), Gentamicin (generic), Tobramycin (generic), Amoxicillin (generic), Amoxicillin/Clavulanate (Augmentin), Amphotericin B (Fungizone), Ampicillin (generic), Atovaquone (Mepron), Cefazolin (generic), Cefepime (Maxipime), Cefotaxime (Claforan), Cefuroxime (Zinacef), Chloramphenicol (generic), Clotrimazole (Mycelex), Ciprofloxacin (Cipro), Clarithromycin (Biaxin), Dicloxacillin (generic), Doxycycline (generic), Erythromycin lactobionate and other salts Fluconazole (Diflucan), Foscarnet (Foscavir), Ganciclovir, (Cytovene, DHPG),

and related compounds, including florphenicol thiamphenicol, and any combination thereof.

Typically, antibiotics active against *Selenomonas ruminantium,* and *Clostridium*-like *vitulinus* can be used to prevent acid accumulation in the gut during carbohydrate fermentation.

Typically, the antibiotics active lactic acid producing microorganisms may be used in conjunction with the vaccine in accordance with the third or twentieth embodiments of the invention.

Typically, the antibiotics active against gram-positive lactic acid producing microorganisms may be used in conjunction with the vaccine in accordance with the third or twentieth embodiments of the invention. For example animals may be immunised against *Selenomonas* type bacteria and fed diets containing the antibiotic virginiamycin active against *Streptococcus* spp. and lactobacilli of lactic acid producing bacteria.

Typically, vaccines against Gram positive lactic acid producing bacteria can be used in combination with the vaccine in accordance with the third or twentieth embodiments of the invention. For example, animals may be immunised against the Gram negative *Selenomonas* type bacteria and against *Streptococcus* spp. and *Clostridium*-like (*Lactobacillus*-like) lactic acid producing bacteria.

Typically, vaccines against Gram positive lactic acid producing bacteria may also be used in combination with vaccines against Gram negative lactic acid producing bacteria, and these vaccines can also be used in conjunction with antibiotic compounds active against lactic acid bacteria.

Typically, the enzyme preparation is active against lactic acid producing gram-negative bacteria. More typically, enzyme preparations are designed to reduce the passage of fermentable carbohydrate to the hind gut through improving the digestion and absorption in the intestine of starches, disaccharides, oligosaccharides, non-starch polysaccharides, protein starch complexes and any polysaccharide which is incompletely digested in the intestine, but which is readily fermentable in the hind gut.

Typically preferred enzymes for the break down of non-starch polysaccharides and starches include the following: glyconases including: amylase,

Typically, the clay preparation active against lactic acid accumulation from gram-negative lactic acid producing microorganisms may be used in conjunction with the vaccine in accordance with the third or twentieth embodiments of the invention.

Typically, the compounds which slow digesta flow rate are indirectly active against lactic acid producing gram-negative bacteria. More typically, by administering compounds which slow digesta flow rate, intestinal digestion and absorption are increased, reducing the amount of fermentable substrate passing to the hind gut.

Generally, preferred agents to slow the flow of digeseta include biologically active peptides (BAP) in a form which will reach the duodenum, and are active in modulating the activity of the digestive tract, gastric emptying and the rate of passage through the intestine. More typically, these biologically active peptides include opioid peptides. Compounds active on the autonomic nervous system (eg atropine and atropine-like compounds) may affect digesta flow and have similar effects. Compounds such as 5HT agonists/antagonists, motilin antagonists, NO promoters, dopamine agonists may also be used.

Whilst a range of proteins potentially produce opioid peptides on hydrolisation, the β-casomorphins, which can be derived from β-casein increasing casein during β-casein digestion, are particularly active.

Even more typically, the biologically active peptides include cholecystokinin (CCK), the M1 fraction of virginiamycin and the analogue of virginiamycin fraction M1, compound L-156. These biologically active peptides can be used individually or in combination.

It has traditionally been assumed that the nutritional benefits of proteins are only related to the essential amino acids supplied to the animal during digestion and absorption. However through the supply of biologically active peptides and the production of naturally occurring opioid peptides, the rate of digesta passage is reduced and this results in more efficient intestinal digestion and less fermentable substrate passing to the hind gut which can contribute to acidic gut syndrome.

Practical methods of supplying biologically active opioid peptides is through dietary supplementation with proteins such as casein and blood meal. For ruminant

ruminantium can ferment sugars or starch without accumulation of lactic acid and these strains can be used to reduce lactic acid accumulation.

More typically, the probiotic preparations may include bacteria that belong to the genera: *Succinomonas, Butyrivibrio, Bacteroides and Succinivibrio*. These bacteria can be used individually or in combination. More typically, the probiotic preparations may include anaerobic bacteria. Even more typically, the probiotic preparations may include bacteria selected from the group consisting of: *Megasphera, Veillenolla, Selenomonas, Propionibacterium, Anaerovibrio* and *Peptococcus*. These bacteria can be used individually or in combination. Still more typically, preferred probiotic preparations include yeast and mycelial preparations capable of utilising lactic acid, and converting lactic acid to volatile fatty acids and other end products. Yet still more typically, the probiotic preparations may include yeast and mycelial preparations such as Yea-Sacc.

Typically, at least any two of the above sample microorganisms of the probiotic preparation may be used in combination in the probiotic preparation.

Typically, the above probiotics may be used in conjunction with the vaccine in accordance with the third or twentieth embodiments of the invention.

Typically, a combination of immunisation/antibiotic treatment, together with the use of probiotics allows the control harmful lactic acid producing bacteria and thus allowing the better establishment of favourable starch utilising organisms. The effect of this combination of treatments is synergistic and not merely additive.

3. Nucleic acid molecules and antibodies

According to a fifteenth embodiment of the invention, there is provided an isolated and purified nucleic acid molecule comprising a polynucleotide sequence capable of selectively hybridising to at least a portion of the nucleic acid of at least one of the isolated and purified microorganisms as defined in accordance with the first embodiment of the invention.

According to a sixteenth embodiment of the invention, there is provided an isolated and purified nucleic acid molecule comprising a polynucleotide sequence selected from the group consisting of: SEQ ID Nos 1-9.

Typically, the nucleic acid molecule corresponds to a DNA or RNA molecule.

performed in conditions of low temperature and/or high salt. Even more typically, low stringency hybridisation conditions correspond to hybridisation performed at 50^{o}C in 6xSSC.

For example, suitable experimental conditions for determining whether a given nucleic acid molecule hybridises to a specified nucleic acid may involve following the following hybridisation routine: presoaking of a filter containing a relevant sample of the nucleic acid to be examined in 5 x SSC for 10 min, and prehybridisation of the filter in a solution of 5 x SSC, 5 x Denhardt's solution, 0.5% SDS and 100 μg/ml of denatured sonicated salmon sperm DNA, followed by hybridisation in the same solution containing a concentration of 10 ng/ml of a ^{32}P-dCTP-labeled probe for 12 hours at approximately 45°C, in accordance with the hybridisation methods as described in Sambrook *et al.* (1989; Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbour, New York).

The filter is then washed twice for 30 minutes in 2 x SSC, 0.5% SDS at least 55°C (low stringency), at least 60°C (medium stringency), at least 65°C (medium/high stringency), at least 70°C (high stringency), or at least 75°C (very high stringency). Hybridisation may be detected by exposure of the filter to an X-ray film.

Further, there are many conditions and factors, well known to those skilled in the art, which may be used to alter the stringency of hybridisation. For instance, alterations features such as: the length and nature (DNA, RNA, base composition) of the nucleic acid to be hybridised to a specified nucleic acid; concentration of salts and other components, such as the presence or absence of formamide, dextran sulfate, polyethylene glycol etc; and altering the temperature of the hybridisation and/or washing steps, all influence the dynamics and stringency of nucleic acid hybridisation.

Further, it is also possible to theoretically predict whether or not two given nucleic acid sequences will hybridise under certain specified conditions. Accordingly, as an alternative to the empirical method described above, the determination as to whether an analogous nucleic acid sequence will hybridise to the nucleic acid molecule in accordance with the fifteenth or sixteenth embodiments of

about 10 to about 75 nucleotides in length. Even more typically, the oligonucleotide fragment is between about 15 to about 50 nucleotides in length. Even more typically still, the oligonucleotide fragment is between about 15 to about 30 nucleotides in length. Yet still more typically, the oligonucleotide fragment is between about 5 to about 25 nucleotides in length.

According to a seventeenth embodiment of the invention, there is provided a vector comprising the nucleic acid molecule as defined in accordance with the fifteenth or sixteenth embodiments of the invention.

Typically, the vector is a shuttle or expression vector. More typically, the vector is selected from the group consisting of: viral, plasmid, bacteriophage, phagemid, cosmid, bacterial artificial chromosome, and yeast artificial chromosome. More typically, the vector is a plasmid and may be selected from the group consisting of: pBR322, M13mp18, pUC18 and pUC19. Even more typically, the vector is a bacteriophage and may be selected from λgt10 and λgt11 or phage display vectors.

According to an eighteenth embodiment of the invention, there is provided a host cell transformed with the vector as defined in accordance with the fourteenth embodiment of the invention.

Typically, the host cells are procaryotic or eucaryotic in nature. More typically, the procaryotic host cells include bacteria, and examples of such bacteria include: *E. coli, Bacillus, Streptomyces, Pseudomonas, Salmonella,* and *Serratia*.

More typically, the eucaryotic host cells may be selected from the group consisting of: yeast, fungi, plant, insect cells and mammalian cells, either *in vivo* or in tissue culture. Examples of mammalian cells include: CHO cell lines, COS cell lines, HeLa cells, L cells, murine 3T3 cells, c6 glioma cells and myeloma cell lines.

According to a nineteenth embodiment of the invention, there is provided an antibody raised against at least one of the following:

(a) at least one fermentative lactic acid producing microorganism in accordance with the first embodiment of the invention;

(b) intact cells of least one fermentative lactic acid producing microorganism in accordance with the first embodiment of the invention;

(m) cell lysate from *Streptococcus bovis* (strain Sb-5) deposited with the Australian Government Analytical Laboratories (AGAL) on 8 March 1994, and given accession number N94/8255;

(n) crude antigen mixture or purified antigen or antigens from *Streptococcus bovis* (strain Sb-5) deposited with the Australian Government Analytical Laboratories (AGAL) on 8 March 1994, and given accession number N94/8255;

(o) outer membrane and associated proteins of *Streptococcus bovis* (strain Sb-5) deposited with the Australian Government Analytical Laboratories (AGAL) on 8 March 1994, and given accession number N94/8255.

Typically, the antibodies in accordance with the nineteenth embodiment of the invention can be comprised of a polyclonal mixture, or may be monoclonal in nature. Further, antibodies can be entire immunoglobulins derived from natural sources, or from recombinant sources. The antibodies of the present invention may exist in a variety of forms, including for example as a whole antibody, or as an antibody fragment, or other immunologically active fragment thereof, such as complementarity determining regions.

According to a twentieth embodiment of the invention, there is provided a vaccine comprising at least one of the antibodies as defined in accordance with the nineteenth embodiment of the invention, together with a pharmaceutically acceptable carrier, adjuvant and/or diluent.

4. Diagnosis of Acidosis

According to a twenty-first embodiment of the invention, there is provided a diagnostic kit for the detection of microorganisms having a role in lactic acidosis in a vertebrate, said kit comprising at least one of the antibodies as defined in accordance with the nineteenth embodiment of the invention, together with a diagnostically acceptable carrier and/or diluent.

Typically, the diagnostic kit may also contain antibodies capable of detecting at least one lactic acid producing strain selected from *Streptococcus* spp., *Selenomonas ruminantium* or lactobacilli, *Clostridium*-like *vitulinus*. The kits will also include analytical methods to measure the extent of acid production, specifically

More typically, the kit may further comprise one or more other containers, containing other components, such as wash reagents, and other reagents capable of detecting the presence of bound antibodies. Even more typically, the detection reagents may include: labelled (secondary) antibodies, or where the antibody (or fragment thereof) in accordance with the nineteenth embodiment of the invention is itself labelled, the compartments may comprise antibody binding reagents capable of reacting with the labelled antibody (or fragment thereof) of the present invention.

According to a twenty-second embodiment of the invention, there is provided a method for screening for the presence of microorganisms having a role in acidosis in a vertebrate comprising

(a) contacting a sample from the gut of a vertebrate with the antibody (or fragment thereof) as defined in accordance with the nineteenth embodiment of the invention, and

(b) detecting the presence of the antibody (or fragment thereof) bound to microorganisms having a role in acidosis.

Typically, the antibody used in the method in accordance with the twenty-second embodiment of the invention corresponds to an antibody mix, comprising antibody or fragment thereof as defined in accordance with the nineteenth embodiment of the invention, together with an antibody(s) selected from the group consisting of: antibodies capable of detecting at least one lactic acid producing strain selected from *Streptococcus bovis* or lactobacilli and antibodies capable of detecting *Streptococcus bovis* (strain Sb-5) deposited with the Australian Government Analytical Laboratories (AGAL) on 8 March 1994, and given accession number N94/8255.

According to a twenty-third embodiment of the invention, there is provided a method for screening for the presence of microorganisms having a role in acidosis in a vertebrate comprising

(a) contacting a nucleic acid sample from a microorganism isolated from the gut of a vertebrate with a nucleic acid probe, and

(b) detecting hybridisation between the nucleic acid sample and the polynucleotide sequence.

-myasthenia gravis, irritable bowel syndrome, crohn's disease, effects on the pancreas, kidneys, thyroid and other organs of the endocrine system, and immune conditions associated with localised inflammation of sections of the gut; homeostasis disorders, including mineral and electrolyte imbalances, such as osteoporosis; impaired reproductive performance; predisposition to ulceration of the gastrointestinal tract; respiratory tract disorders, including asthma; hypertension; infected gums and dental caries; viral infections, including herpes; predisposition to infection by bacteria, viruses and mycoplasmas fungi or protozoa; exacerbation of heat stress, and impaired hair, milk production and wool growth.

Definitions

The term "antibody" means an immunoglobulin molecule able to bind to a specific epitope on an antigen. Antibodies can be comprised of a polyclonal mixture, or may be monoclonal in nature. Further, antibodies can be entire immunoglobulins derived from natural sources, or from recombinant sources. The antibodies of the present invention may exist in a variety of forms, including for example as a whole antibody, or as an antibody fragment, or other immunologically active fragment thereof, such as complementarity determining regions. Similarly, the antibody may exist as an antibody fragment having functional antigen-binding domains, that is, heavy and light chain variable domains. Also, the antibody fragment may exist in a form selected from the group consisting of: Fv, F_{ab}, $F(ab)_2$, scFv (single chain Fv), dAb (single domain antibody), bi-specific antibodies, diabodies and triabodies.

The antibody (or fragment thereof) in accordance with the nineteenth embodiment of the present invention has binding affinity to a microorganisms capable of producing acid in the gut of vertebrates in vertebrates. Preferably, the antibody (or fragment thereof) has binding affinity or avidity greater than about 10^5 M^{-1}, more preferably greater than about 10^6 M^{-1}, more preferably still greater than about 10^7 M^{-1} and most preferably greater than about 10^8 M^{-1} .

The techniques for generating and reviewing binding affinity are reviewed in Scatchard (1949), Annals of the New York Academy of Sciences, 51, 660-672, and Munson (1983), Methods in Enzymology 92, 543-577, the contents of each of which are incorporated herein by reference.

the outer membrane and associated proteins in a pharmaceutically/veterinarily acceptable carrier, diluent, excipient and/or adjuvant.

Oral vaccination can then be performed by delivering the dead cells of the microorganisms and/or outer membrane and associated proteins, in a biodegradable polymer capsule, composed of gelatine or the like, or an appropriate slow release device(s) are well known in the art. The vaccine can also be suitably diluted to a manageable volume with an appropriate pharmaceutically/veterinarily acceptable carrier, diluent and/or adjuvant and delivered orally using devices such as those used for the delivery of anthelmintics, for example.

In delivery systems utilising the parenteral route it is preferred that dead cells of the microorganisms and/or outer membrane and associated proteins, are suitably washed, harvested and resuspended in a pharmaceutically/veterinarily acceptable carrier, diluent and/or adjuvant suitable for injection, utilising methods of administration as are well known in the art.

In the administration of therapeutic formulations in accordance with the present invention and herein disclosed, there are preferred non-toxic pharmaceutical carriers, diluents, excipients and/or adjuvants. For administration of the above formulations the microorganism or fragment or fragments thereof of the present invention are admixed with these non-toxic carriers, diluents, excipients and/or adjuvants and may be in the form of capsules, aqueous or oily suspensions, emulsions, syrups, elixirs, micelles or injectable solutions.

Examples of pharmaceutically and veterinarily acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters

paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

Further, a vaccine composition containing a recombinant polypeptide as encoded by at least one of the nucleic acid molecules in accordance with the fifteenth or sixteenth embodiments of the invention, may be prepared for use by standard methods, well known to those of ordinary skill in the art.

In one embodiment, the immunogenic polypeptide, glycopeptide or the like, may be produced in a recombinant system by expression of the polynucleotide sequence (or a fragment thereof) in accordance with the fifteenth or sixteenth embodiments of the invention, and subsequently isolated. For example, microbial cells containing the nucleic acid molecule of interest may be cultured in large volume bioreactors, then collected by centrifugation and subsequently ruptured, for instance by high-pressure homogenisation. The resulting cell lysate may be resuspended in appropriate diluent such as those described herein, and filtered to obtain an aqueous suspension of the immunogen. The recombinant protein can be administered in crude form, for example, by diluting in a 0.1M phosphate buffer (pH 7.4) to 50-500 μg/ml concentration, and then passing through a sterile 0.22 micron filter.

control sequences, such as an origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

A typical vaccination regime is to deliver the vaccine in multiple doses generally one, two or three equal doses.

In general to induce the production of antibodies to the vaccines of the invention, they can be oleogenous or aqueous suspensions formulated in accordance with known methods in the art using suitable dispersing, suspension and/or wetting agents. Examples of suitable dispersing, suspension and wetting agents include Freund's complete/incomplete adjuvant, Montenide Marcol adjuvant and phosphate buffered saline, and mannan.

It will be appreciated that the examples referred to above are illustrative only and other suitable carriers, diluents, excipients and adjuvants known to the art may be employed without departing from the spirit of the invention.

The vaccines of the invention are typically formulated for administration by an oral or parenteral route, by inhalation or topical. The term parenteral as used herein includes intravenous, intradermal, intramuscular, subcutaneous, rectal, vaginal or intraperitoneal administration. The subcutaneous and intramuscular forms of parenteral administration are generally preferred.

The parenteral and oral dosage regimens for employing compounds of the invention to prophylactically or therapeutically control lactic acidosis will generally be in the range of about 0.01 to about 100, preferably about 0.01 to about 50, more preferably about 0.05 to about 25, even more preferably about 0.1 to about 2 milligrams per kilogram body weight per day. Alternatively, dosage rates can be determined in relation to metabolic rate or surface area of the body.

A vaccine or pharmaceutical composition of the invention may also be administered by inhalation, that is, intranasal and/or oral inhalation administration. Appropriate dosage forms for such administration, such as an aerosol formulation or a metered dose inhaler, may be prepared by conventional techniques. The preferred dosage amount of a compound of the invention to be employed is generally within the range of about 0.05 to about 100, preferably about 0.05 to about 50, more preferably about 0.5 to about 25, even more preferably about 0.5 to about 10 milligrams per kilogram body weight per day.

Typically, the injection volume for sheep is between 1 mL to 3 mL, and 2 to 7 mL for cattle and horses 3 to 5 mL. More typically, the injection volume for sheep is between 1 mL to 2 mL, and 1 to 5 mL for cattle and horses.

In accordance with any one of the twelfth through to fourteenth embodiments of the invention, the administered dose of the antibiotic can vary and will depend on several factors, such as the condition, age and size of the human or animal patient, as well as the nature of the lactic acid producing bacteria.

Dosages will typically range from between any one of the following: 0.01 and 100 mg per kg of bodyweight; 0.01 and 75 mg per kg of bodyweight; 0.01 and 50 mg per kg of bodyweight; 0.01 and 25 mg per kg of bodyweight; 0.01 and 15 mg per kg of bodyweight; 0.01 and 10 mg per kg of bodyweight; and 0.01 and 5 mg per kg of bodyweight. More typically dosages will range from between 0.02 and 2.0 mg per kg of bodyweight. More typically dosages will range from between 0.05 and 1.0 mg per kg of bodyweight. Even more typically dosages will range from between 0.1 and 0.5 mg per kg of bodyweight. Yet even more typically, the antibiotic is administered to the human or animal at a rate of 0.4 mg per kg of bodyweight.

Typically, the antibiotic is administered at a rate of between 1 and 100 mg per kg of dry weight of food. More typically, the antibiotic is administered at a rate of between 1 and 75 mg per kg of dry weight of food. Even more typically, the antibiotic is administered at a rate of between 1 and 50 mg per kg of dry weight of food. Yet even more typically, the antibiotic is administered at a rate of between 5 and 40 mg per kg of dry weight of food.

Typically, antibiotic preparations are selected and/or formulated for delivery to the hind gut and for little or no absorption from the digestive tract. Formulations include encapsulation and/or coating with materials resistant to acid and enzymic digestion in the stomach and small intestine. Formulation can also include chemical treatment to reduce the solubility of the antibiotic.

As above, the administered dose of the enzyme preparation can vary and will depend on several factors, such as the condition, age and size of the human or animal patient, as well as the nature of the carbohydrate. Dosages will typically range from between 0.01 and 50 g/kg food dry matter. Typically, the enzyme is

food and animal feeds. They can be fed as powders or suspended in water, included in pellets as well as being fed in premixes.

More typically the active agent is mixed with the food, or is added to feeds which contain starch or sugars which may produce an acidic pattern of fermentation in the gastrointestinal tract. The active agent can also be added to water included in tablets and the like.

A suitable treatment may include the administration of a single dose or multiple doses. Usually, the treatment will consist of administering one dose daily of the active agent for a period sufficient to control the accumulation of acid by fermentation of the carbohydrate in the gastrointestinal tract. Dosing may continue while sources of carbohydrate known to cause problems of acidic fermentation in the gastrointestinal tract are included in the diet.

More typically the active agent may be administered in a single dose immediately before consuming meals containing sources of carbohydrate which are poorly digested and rapidly fermented.

More typically, the active agent is administered for one day prior to and daily during the consumption of excessive quantities of food stuffs containing readily fermentable carbohydrates.

Typically, the active agent is administered orally.

2. Antibodies

Antibodies or immunoglobulins are typically composed of four covalently bound peptide chains. For example, an IgG antibody has two light chains and two heavy chains. Each light chain is covalently bound to a heavy chain. In turn each heavy chain is covalently linked to the other to form a "Y" configuration, also known as an immunoglobulin conformation. Fragments of these molecules, or even heavy or light chains alone, may bind antigen.

A normal antibody heavy or light chain has an N-terminal (NH_2) variable (V) region, and a C-terminal (COOH) constant (C) region. The heavy chain variable region is referred to as V_H (including, for example, $V\gamma$), and the light chain variable region is referred to as V_L (including $V\kappa$ or V_λ). The variable region is the part of the molecule that binds to the antibody's cognate antigen, while the Fc

isotype is found in pentameric form; secreted IgA can be found in both monomeric and dimeric form.

In a related aspect, the invention features a monoclonal antibody, or an Fab, $(Fab)_2$, scFv (single chain Fv), dAb (single domain antibody), bi-specific antibodies, diabodies and triabodies, or other immunologically active fragment thereof (eg., a CDR-region). Such fragments are useful as immunosuppressive agents. Alternatively, the antibody of the invention may have attached to it an effector or reporter molecule. For instance, an antibody or fragment thereof of the invention may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. In addition, the Fc fragment or CH_3 domain of a complete antibody molecule may be replaced or conjugated by an enzyme or toxin molecule, such as chelates, toxins, drugs or prodrugs, and a part of the immunoglobulin chain may be bonded with a polypeptide effector or reporter molecule, such as biotin, fluorochromes, phosphatases and peroxidases. Bispecific antibodies may also be produced in accordance with standard procedures well known to those skilled in the art.

The present invention further contemplates genetically modifying the antibody variable and/or constant regions to include effectively homologous variable and constant region amino acid sequences. Generally, changes in the variable region will be made to improve or otherwise modify antigen binding properties of the antibody or fragment thereof. Changes in the constant region will, in general, be made in order to improve or otherwise modify biological properties, such as complement fixation, interaction with membranes, and other effector functions.

Typically, the antibodies in accordance with the nineteenth embodiment of the invention can be comprised of a polyclonal mixture, or may be monoclonal in nature. Further, antibodies can be entire immunoglobulins derived from natural sources, or from recombinant sources. The antibodies of the present invention may exist in a variety of forms, including for example as a whole antibody, or as an antibody fragment, or other immunologically active fragment thereof, such as complementarity determining regions.

Monoclonal antibodies can be obtained by various techniques familiar to those skilled in the art. For example. spleen cells from an animal immunised with a

Conditions for incubating an antibody (or fragment thereof) with a test sample vary widely, depending on the format of detection used in the assay, the detection method, and the type and nature of the antibody used. A person of ordinary skill in the art would readily appreciate that any one of the commonly available immunological assays could be used in performing the method of detection. For example, these assays include: radioimmunoassays, enzyme-linked immunosorbent assays, and/or immunoflourescent assays.

A kit for performing the above method of the invention contains all the necessary reagents to carry out the above methods of detection. For example, the kit may comprise the following containers:

(a) a first container containing the antibody (or fragment thereof) in accordance with the nineteenth embodiment of the present invention;

(b) a second container containing a conjugate comprising a binding partner of the antibody (or fragment thereof), together with a detectable label.

Typically, the kit may further comprise one or more other containers, containing other components, such as wash reagents, and other reagents capable of detecting the presence of bound antibodies. More typically, the detection reagents may include: labelled (secondary) antibodies, or where the antibody (or fragment thereof) of the present invention is itself labelled, the compartments may comprise antibody binding reagents capable of reacting with the labelled antibody (or fragment thereof) of the present invention.

Further, the kit of the present invention, as described above in relation to antibodies, can be readily incorporated, without the expenditure of inventive ingenuity, into a kit for nucleic acid probes. One skilled in the art would select the nucleic acid probe from the polynucleotides of the present invention, according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of vertebrate tissue.

Such a kit comprises at least one container means having disposed therein the above-described nucleic acid probe. The kit may further comprise other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatic labelled probes

rumen fistula from cattle 24 h after regular grain feeding. Faecal samples were obtained directly from the rectum of sheep and cattle and from the freshly voided faeces from horses. Samples were processed for the enumeration of lactic acid bacteria following the method of Yanke and Cheng (1998) involving one-hour exposure prior to incubation. A semi-selective MRS-agar medium, Oxoid, England (De Man *et al.* 1960) was modified by adding a freshly prepared reducing solution (1ml containing 0.026g Cysteine, HCl and 0.026g $Na_2S.9H_2O$ per 100 ml of media) after boiling then pre-reduced by bubbling with CO_2 on ice until cold. The pH of the medium was adjusted to 5.5 during preparation. Viable colonies from roll tubes were picked and inoculated into a broth of a basal medium 10 (BM 10) as described by Caldwell and Bryant (1966) with glucose (0.5%) and then again cultured in roll tubes. The procedure of picking colonies and inoculating them into a broth medium followed by inoculating again into roll tubes was repeated twice. At 48 h of incubation a drop of the broth medium was examined under the microscope to check the purity of the culture. Morphology and Gram staining characteristics of the isolates were recorded. The ability of cultures to ferment various carbohydrates was evaluated using a broth of BM10 with each isolate included at 2g/L. *S. bovis* was distinguished from *S. equinus* on the basis of their ability to ferment starch, inulin and lactose and their ability to survive heating to 60°C for 30 minutes was also tested (Hardie 1986).

Fermentation products were measured after 24 h of anaerobic incubation of a broth consisting of BM 10 with glucose or starch (0.5%) at 39°C. At the end of the fermentation period samples from the media were taken for measurement of pH, then acidified with sulphuric acid for further analysis of VFA and lactate. VFA concentrations were measured using a gas chromatograph (Packard Model 427, Packard Instrument Company, Inc, Illinios, USA), fitted with a Chromosorb 'W', acid washed and 60-80 mesh column coated with two liquid phases, a: o-phosphoric acid (1.5% w/w) and b: Polypropylene glycol sebacate (17.5% w/w). The temperature for the column, detector and the injector were 135, 180 and 210°C respectively. L-lactate and D-lactate were analyzed by auto-analyzer (Cobas Mira Autoanalyser, Roche Diagnostics Inc., French Forest, NSW) using an enzymatic procedure (Stat-Pack ™ Rapid Lactate Test, Cat. No. 1112 821, Behring

-ng of each primer, 10μl of 10X buffer, 6μL of $MgCl_2$, 1U of Tth DNA polymerase (Biotech International, Perth, Australia), 10μL of 4x0.5mM dNTP's, and the remainder made up with sterile Milli-Q water and 2μL concentrated cell suspension or DNA extract. Reactions were overlaid with sterile mineral oil and carried out in a thermocycler (Perkin-Elmer DNA Thermal Cycler 480). Thermocycling parameters employed after a 96°C denaturation for 10 min were 28 cycles of 1 min at 94°C, 1 min at an annealing temperature, and 2 min at 72°C. A further extension step involving 1 min at 48°C and 5 min at 72°C was also employed. The primers used were 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-GGTTACCTTGTTACGACT-3') (Lane 1991). In some cases GeneReleaser (Bioventures, Inc., Tennessee, USA) was used according to the manufacturer's instructions in the reactions outlined above. PCR products were purified using a QIAquick PCR purification kit (QIAGEN, Victoria, Australia) according to the manufacturer's instructions.

Sequencing of 16S rDNA

All 16S rDNA samples were initially partially sequenced using the universal 16S rRNA primer, 530f (5'-GTGCCAGCMGCCGCGG-3') and an ABI Big Dye Terminator Cycle Sequencing Ready Reaction Mix kit (Victoria, Australia). Selected 16S rDNA were subsequently fully sequenced on both strands using the following primers:519r (5'-GWATTACCGCGGCKGCTG-3'), 27f, 907r (5'-CCGTCAATTCMTTTRAGTT T-3'),

926f (5'-AAACTYAAAKGAATTGACGG-3'), and 1492r (Lane, 1991).

Approximately 100 ng of purified PCR product and 25 ng of primer were used in the sequencing reactions. Thermal cycling was carried out in an MJ Research PTC-100 thermocycler with an initial denaturation step of 96°C for 2 min, followed by 25 cycles of 50°C for 15s, 60°C for 4 min, and 96°C for 30s. The resulting cycle sequencing products were purified using the ethanol plus sodium acetate method (ABI, Australia). Purified sequencing products were submitted to the Australian Genome Research Facility for analysis on an Applied Biosystems 377 automated sequencer.

or xylose and two of the white pigmented isolates did not ferment inulin (Table 2). L-lactate was the main fermentation product of *S. bovis* and *S. equinus*.

The *Clostridium*-like *vitulinus*-like isolates have 100% sequence identity to each other, and 97% identity with *Clostridium*-like *vitulinus*. *L vitulinus*-like cells occurred in different shapes and sizes, mainly straight rods in singles, pairs and short chains and some cells have the tendency to branch. *Clostridium*-like *vitulinus* isolates could not ferment starch or xylose but grew on cellobiose, fructose, glucose, mannose, raffinose and sucrose (Table 2). Four R. vitulinus isolates produced D-lactate and one produced L- and D-lactate at equal proportions.

S. ruminantium was isolated from the rumen of grass-adapted sheep that received a grain supplement with or without virginiamycin. The three *Selenomonas*-like isolates formed two distinct lines of descent: Type A (isolate SR R1); and Type B (isolate SR R2 and isolate SR R3). Type A has 99% identity to *S. ruminantium* and >97% identity to Type B, and Type B has >97% identity to *S. ruminantium*. This indicates that all three isolates are members of the genus *Selenomonas* and Type A belongs to the species *S. ruminantium*. One isolate (SR R1) produced L-Lactate while the other two (SR R2 and SR R3) produced L- and D-lactate (Table 3). A difference between SR R2 and SR R3 was that SR R3 produced 42.5% more lactic acid from starch than SR R2. *S. ruminantium* grew on most carbohydrate sources (Table 2) but their growth was noticeably high on glucose and sucrose compared with other sources and only one isolate (SR R1) did not ferment starch. The cells are 0.5-0.7 × 1.5-3.0 μm, occurred mainly in single short crescent rods, with round ends and Gram-negative. Similar amounts of L-lactate were produced from glucose, starch or raftilose by the different *S. bovis* isolates (Table 4).

Incubation *in vitro* of *S. bovis*, *Clostridium*-like *vitulinus* and *S. ruminantium* isolates in a broth of Basal Medium 10 with glucose (0.5%) with different concentration of VM (0, 2, 4, 6 and 8 μg/ml) clearly demonstrated sensitivity of *S. bovis* to virginiamycin. *Clostridium*-like and *Selenomonas* isolates (Table 5) showed varying levels of resistance to VM. LV R1 and LV R5 were very sensitive to VM with a MIC level of 2 μg/ml. Isolates LV R2 and LV R3 resisted levels up to 4 μg/ml and LV R4 up to 6 μg/ml. *S. ruminantium* isolates were resistant to the highest VM levels (8 μg/ml). Sensitivity of *Clostridium*-like bacteria to VM was

Example 2

Samples of faeces were collected from 12 horses fed a mixture of chopped lucerne hay and different types of cereal grain (oats, barley, triticale and sorghum). The dominant lactic acid producing bacteria were isolated using the same method as described in Example 1. Two bacteria were isolated: *Streptococcus bovis* (SbR1) and *Streptococcus equinus* (SER1 and SER2). Based on these results it is important that any vaccine, diagnosis or other treatment to prevent or control acidosis should be effective against both *S. Bovis* and *S. equinus*. Under some conditions it will also be necessary for these diagnostic tools and methods of treatment to be effective against *S. ruminantium*, *Clostridium*-like *vitulinus* as well as *S. Bovis* and *S. equinus*.

In certain conditions it is likely that *Selenomonas ruminantium* and *Clostridium*-like *vitulinus* bacteria may be present and in this case animals should be vaccinated against both Gram-positive and Gram-negative lactic acid producing bacteria.

Example 3

Following calving cows grazing lush green pasture are exposed to high levels of soluble carbohydrates in the form of fructans in grasses and sugars and starch in clovers. In addition to fermentable carbohydrates in the pasture, concentrate feed supplements, based on cereal grain, are fed twice daily during milking. The fructans in pastures and the starch in legumes and concentrate are rapidly fermented in either the rumen or the hind gut to form a range of volatile fatty acids and lactic acid. The accumulation of acids in the gut contribute to the metabolic acid load of the animal, can cause inflammation of the gut wall leading to stimulation of the immune system and can lead to increased pathogenicity in the populations of bacteria and parasites within the gut. The adverse effects of acid accumulation in the gut result in reduced productivity and an increased incidence of disease including lameness, respiratory conditions and mastitis.

Acid accumulation in the gut under this dietary regime can be satisfactorily reduced by controlling two of the principle acid producing bacteria *Selenomonas ruminantium* and *Streptococcus* spp. As one of these bacteria is Gram-negative and the other Gram-positive two approaches are used. A vaccine is used to control

reduced by immunisation against *Streptococcus equinus* and the strategic use of antibiotics active against *Clostridium*-like *vitulinus* and *Selenomonas ruminantium*.

Example 7

Humans suffering from lactose intolerance, irritable bowel syndrome, or any side effects of acidic gut syndrome can be immunised against *Clostridium*-like *vitulinus* and/or *Selenomonas ruminantium* and treated strategically with antibiotics such as virginiamycin and probiotics such as *Megasphera elsdenii* and *Biphidabacter*.

References

Altschul, S.F., Gish W., Miller W., Myers E.W., and Lipman D.J. (1990). Basic local alignment search tool. Journal of Molecular Biology **215**, 403-410.

Bryant M.P. 1956. The characteristics of strains of *Selenomonas* as isolated from bovine rumen contents. Journal of Bacteriology **72**, 162-167.

Caldwell D.R. and Bryant M.P. 1966. Medium without rumen fluid for nonselective enumeration and isolation of rumen bacteria. Applied Microbiology **14**, 794-801.

Courtney D.A. and Seirer R.C. 1996. Supplementary feeding of grain to cattle with virginiamycin to reduce the risk of acidosis. Animal Production in Australia **21**, 344.

De Man J.C., Rogosa M. and Sharp M.E. 1960. A medium for the cultivation of lactobacilli. Journal of Applied Bacteriology **23**, 130-135.

Dojka, M.A., Hugenholtz P., Haack S.K., and Pace N.R. (1998). Microbial diversity in a hydrocarbon- and chlorinated-solvent-contaminated aquifer undergoing intrinsic bioremediation. Applied and Environmental Microbiology **64**, 3869-3877.

Godfrey S.I. Rowe J.B. Speijers E.J. and Toon W. 1993. Lupin, barley, or barley plus virginiamycin as supplement for sheep at different feeding intervals. Australian Journal of Experimental Agriculture **33**, 135-140.

Godfrey S.I., Rowe J.B., Thorniley G.R., Boyce G.R. and Speijers E.J. 1995. Virginiamycin to protect sheep fed wheat, barley or oats from grain poisoning under simulated drought feeding conditions. Australian Journal of Agricultural Research **46**, 393-401.

Hardie J.M. 1986. Other streptococci. In: Bergey's Manual of Systematic Bacteriology. Volume 2. Ed.: Peter H.A. Sneath. pp. 1068-1069. Williams & Wilkins, Baltimore, USA.

Huntington G.B. 1993. Nutritional problems related to the gastro-intestinal tract: Acidosis. In: D.C. Church (Ed.) The ruminant animal, Digestive physiology and nutrition. pp. 474-480. Waveland Press, Inc. Illinois, U. S. A.

Lane, D.J. (1991). 16S/23S sequencing. In: Nucleic Acids Techniques in Bacterial Systematics. Eds.: Stackebrandt, E. and Goodfellow, M. pp. 115-175, John Wiley and Sons, New York.

Yanke L.J. and Cheng K.-J. 1998. A method for the selective enumeration and isolation of ruminal *Lactobacillus* and *Streptococcus*. Letters in Applied Microbiology **26**, 248-252.

Table 2 Fermentation of carbohydrate substrates by *S. bovis*, *S. equinus*, *Clostridium*-like *vitulinus* and *S ruminantium* isolates from pasture-adapted sheep supplemented with wheat grain plus urea.

Carbohydrate	Bacterial Isolates									
	Streptococcus		*Clostridium*-like *vitulinus*					*S. ruminantium*		
	S. bovis	*S. equinus*	LV R1	LV R2	LV R3	LV R4	LV R5	SR R1	SR R2	SR R3
Arabinose	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	+	+	+	-	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	-	-	+	-	-	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Glycerol	-	-	-	-	-	-	-	-	-	-
Inulin	+*	-	+	+	-	+	+	-	-	-
Lactose	+	-**	-	-	+	-	-	+	+	+
Maltose	+	+	+	+	-	+	+	+	+	+
Mannitol	-	-	-	-	+	-	-	+	-	-
Mannose	+	+	+	+	+	+	+	+	+	+
Raffinose	+	+	+	+	+	+	+	+	+	+
Ribose	-	-	-	-	-	-	-	+	+	-
Sorbitol	-	-	-	-	-	-	-	+	+	-
Starch	+	-	-	-	-	-	-	-	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Xylose	-	-	-	-	-	-	-	-	-	+

* Two of the white pigmented *S. bovis* isolates did not ferment inulin; ** one *S. equinus* isolate grew on lactose.

Table 4 Lactate production (mmol/l) by *S. bovis* and *Clostridium*-like *vitulinus* cultures after 24h of anaerobic incubation in a broth of basal medium 10 with glucose or starch added at 0.5% at 39°C. The pH of BM 10 with glucose was 6.92 and with starch was 7.20.

Isolates	*S. bovis** (Sb R1, Sb R2, Sb R3)		*C*-like *vitulinus* (LV R1, LV R2, LV R4, LV R5)		*C*-like. *vitulinus* (LV R3)	
Carbohydrate	Glucose	Starch	Glucose	Starch	Glucose	Starch
L-Lactate	41.1 (± 0.22)	42.8 (± 0.50)	0.0	0.0	20.7	0.0
D-Lactate	0.0	0.0	39.3 (±1.87)	0.0	20.6	0.0
Total (mmol/l)	41.1 (± 0.22)	42.8 (± 0.50)	39.3 (±1.87)	0.0	41.3	0.0
Final pH	5.04 ± 0.001	5.24 ± 0.02	5.57± 0.07		5.53	

* Mean values ± SE.

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~~James Baber Rowe~~
~~R.A.M. Al Jassim~~
The University of New England
Patent Attorneys for the Applicants/Nominated Persons
SPRUSON & FERGUSON

FIGURE 1

SEQ ID NO:1
Streptococcus bovis **(Sb R1) / A2**

CCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAG
TTAAAGGCAGTGGCTTAACCATTGTTCGCT
TTGGAAACTGTTAGACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTG
TAGCGGTGAAATGCGTAGATATATGGAGGA
ACACCGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCG
AAAGCGTGGGGAGCAAACAGGATTAGATAC
CCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCG
GGGCTTAGTGCCGCAGCTAACGCATTAAG
CACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGG
TTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATG
CTATTCCTAGAGATAGGAAGTTTCTTCGGA
ACATCGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCC
CTATTGTTAGTTGCCATCATTAAGTTGGGCACTCTAGCGAGACTGCCGGTA
ATAAACCGGAGGAAGGTGGGGATGACGTC
AAATCATCATGCCCCTTATGACCTGG

Fig 1B

SEQ ID NO:3

Streptococcus equinus **(SE R2) / H15.13**

ATTTATTGGGCGTAAAGCGAGCGCAGGCGGTTTAATAAGTCTGAAGTTAA
AGGCAGTGGCTTAACCATTGTTCGCTTTGG
AAACTGTTAGACTTGAGTGCAGAAGGGGAGAGTGGAATTCCATGTGTAGC
GGTGAAATGCGTAGATATATGGAGGAACAC
CGGTGGCGAAAGCGGCTCTCTGGTCTGTAACTGACGCTGAGGCTCGAAAG
CGTGGGGAGCAAACAGGATTAGATACCCTG
GTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGGCCCTTTCCGGGG
CTTAGTGCCGCAGCTAACGCATTAAGCACT
CCGCCTGGGGAGTACGACCGCAAGGTTGAAACTCAAAGGAATTGACGGG
GGCCCGCACAAGCGGTGGAGCATGTGGTTTA
ATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCGATGCTAT
TTCTAGAGATAGGAAGTTTCTTCGGAACAT
CGGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCCTAT
TGTTAGTTGCCATCATTAAGTTGGGCACTCTAGCGAGACTGCCGGTAATAA
ACCGGAGGAAGGTGGGGATGACGTCAAAT
CATCATGCCCCTTATGACCTGG

Fig 1D

SEQ ID NO:5
***Clostridium*-like *vitulinus* (RV R4) / 111.6**

GATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAACGGAATCTTC
GGATTCCAGTGGCGAACGGGTGAGGAATAC
ATAGGTAACCTGCCCCTCCGAGGGGGACAACAGACGGAAACATCTGCTAA
GACCGCATAGCCACAGGGAAGGCATCTTCC
CTGTGCCAAATGTCCTTTCGGGGACAGCGGGGGGATGGACCTATGCCGCA
TTAGCTGGTTGGCGGGGCAACGGCCCACCA
AGGCGACGATGCGTAGCCGGCCTGAGAGGGCGGACGGCCACACTGGGAC
TGAGACACGGCCCAGACTCCTACGGGAGGCA
GCAGTAGGGAATTTTCGGCAATGGGGGAAACCCTGACCGAGCAACGCCGC
GTGAACGATGAAGGCCTTCGGGTCGTAAAG
TTCTGTTGCGAAGGAAGAACGCCGGTGTCAGGAAATGGGCGCCGGGTGAC
GGTACTTCGCATAGAAAGCCACGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCGAGCGTTATCCGGAATC
ATTGGGCGTAAAGAGGGAGCAGGCGGCGAT
ACAGGTCTGTGGTGAAATTCCGAAGCTAAACTTCGGCCAGCCAAAGAAAC
CGGATCGCTAGAGTGCGGAAGAGGATCGTG
GAATTCCATGTGTAGCGGTGAAATGCGTAGATATATGGAGGAACACCAGT
GGCGAAGGCGACGGTCTGGGCCGCAACTGA
CGCTCATTCCCGAAAGCGTGGGGAGCAAATAGGATTAGATACCCTAGTAG
TCCACGCCGTAAACGATCGATACTAAGTGT
CGGGGGTCAAACCTCGGTGCTGGAGTCAACGCAATAAGTATCGCGCCTGA
GTAGTACGTTCGCAAGAATGAAACTCAAAG
GAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAG
CAACGCGAAGAACCTTACCAGGTCTTGACA
TCGATCCAAAAGGGACGGAGACGTCCCCATAGCTATGGAGAAGACAGGT
GGTGCATGGTTGTCGTCAGCTCGTGTCGTGA
GATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGTCGCCAGTTGCCA
GCATTGAGTTGGGGACTCTGGCGAGACTGC
CTCTGCAAGGAGGAGGAAGGCGGGGATGACGTCAAATCATCATGCCCCTT
ATGACCTGGGCCACACACGTGCTACAATGG
ACGGAGCAGAGGGAAGCGAAGCGGCGACGCCAAGCGGATCCCAGAAACC
CGTTCTCAGTTCGGACTGCAGTCTGCAACTC
GACTGCACGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCTCGGGCCTTGTACACACCGC
CCGTCACACCATGAGAGTCGGCAACACCCGAAGCCGGTGGCTCAACCCCT
CGGGGAGGGAGCTGTCTAAGGTGGGGCCGA
TGATTGGGGTG

Fig 1F

SEQ ID NO:7

***Selenomonas ruminantium* (SR R3) /111.2**

TGGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGGGAGCGCAGGCGGGA
AGGTAAGTCGGTCTTAAAAGTGCGGGGCTCAACCCCGTGATGGGATCGAA
ACTATCTTTCTTGAGTGCAGGAGAGGAAAGCGGAATTCCTAGTGTAGCGG
TGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTTCTG
GACTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTA
GATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTAGGAGGT
ATCGACCCCTTCTGTGCCGGAGTTAACGCAATAAGCATTCCGCCTGGGGA
GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA
GCGGTGGAGTATGTGGTTTAATTCGACGCAACGCGAAGAACCTTACCAGG
GCTTGACATTGAGTGAAAGGGCTAGAGATAGCTCCCTCTCTTCGGAGACA
CGAAAACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCCTATCTTTTGTTGCCAGCACGTCAA
GGTGGGAACTCAAAAGAGACTGCCGCGGACAACGCGGAGGAAGGCGGGG
ATGACGTCAAGTCATCATGCCCCTTATGTCCTGGGCTACACACGTACTACA
ATGGGATGGACAGAGAGCAGCG
AACCCGCGAG

9/10

Fig 1H

SEQ ID NO:9
Selenomonas ruminantium (SR R3)

TGGCGAGCGTTGTCCGGAATTATTGGGCGTAAAGGGAGCGCAGGCGGGA
AGGTAAGTCGGTCTTAAAAGTGCGGGGCTCAACCCCGTGATGGGATCGAA
ACTATCTTTCTTGAGTGCAGGAGAGGAAAGCGGAATTCCTAGTGTAGCGG
TGAAATGCGTAGATATTAGGAGGAACACCAGTGGCGAAGGCGGCTTTCTG
GACTGTAACTGACGCTGAGGCTCGAAAGCGTGGGGAGCGAACAGGATTA
GATACCCTGGTAGTCCACGCCGTAAACGATGAATGCTAGGTGTAGGAGGT
ATCGACCCCTTCTGTGCCGGAGTTAACGCAATAAGCATTCCGCCTGGGGA
GTACGGTCGCAAGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAA
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